Taxonomic study of the family Coccinellidae (Insecta: Coleoptera) from Jammu and Kashmir

PhD Synopsis

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1. Background

Ladybirds, otherwise called ladybugs or ladybird beetles, belong to the family Coccinellidae. These beetles are part of the chitins winged insects (Order: Coleoptera) belonging to the super-family Cucujoidea and under the sub order Polyphaga (Kovar, 1996; Hunt *et al*., 2007). Coccinellidae family has cosmopolitan status, hence the members of Coccinellidae are distributed in most countries of the world. This family comprises 360 genera and approximately 6000 species worldwide (Vandenberg, 2002). Coccinellids are predatory insects which are usually applied as effective biological control agents for insect pest management in various crops (Obrycki and Kring, 1998) however, certain phytophagous species such as *Epilachna* spp. are reported as crop pests (Omkar and Pervez, 2016). These phytophagous species feed on the plants of family Solanaceae (Mishra and yousuf, 2019). Predatory ladybirds play a significant role in development of biological control strategies (Honk and Hodek, 1996). First spectacular success was achieved in 1889 for the control of *Icerya purchasi* Mask, in california by employing the Coccinellid beetle *Rodolia Cardinalis* Mulsant which was imported from Australia. Later, in 1929, it was introduced in India to combat the same species at the Nilgris where the *Icerya purchasi* Mask, had become a serious pest of *Acacia decurrens* Willd, *Acacia dealbata* Link and other plants (Beeson, 1941; Kapur, 1942). *Chilocorus infernalis* Mulsant was tested against Sanjose scale in apple orchards at five locations in farmers fields in kashmir (2008) with a release of four rates of predators which significantly reduced the infestation of the Sanjose scale in all locations of Kashmir (Khan, 2010).

Most of the ladybird species, both adults and larvae, are predators (entomophagous). They feed on small insects such as aphids, whiteflies, scale insects, mealybugs and mites (Moreton, 1969; Hawkeswood, 1987; Majerus, 1994 and Salehi *et al.* 2011). Besides this big group of zoophagous species, ladybird beetles also contain mycophagous species, which includes many serious pests of economically important crops (Giorgi, *et al*. 2009). India is one of the countries that concerns with mass-rearing of Coccinellids, *Coccinella septempunctata* and *Harmonia axyridis* which are used for the control of many pests in field crops (Bianchi and Van der Werf 2003; Koch *et al*. 2005). Coccinellids are rich in species, with ecological diversity of substantial agricultural significance, yet have been consistently problematic to identify and classify with evolutionary relationships poorly understood. Due to

their species richness, morphological, ecological, behavioural diversity and economical significance in agriculture, ladybird beetles have served extensively as model organisms for ecological and evolutionary research (Maredia *et al.* 1992 ; Robertson *et al.* 2008). In order to identify major clads within Coccinellidae, it is worth to evaluate the current classification system, and identify likely drivers of diversification in this polyphagous group. Different classification systems have been used to classify members of the family Coccinellidae depending on the taxonomists and many attempts have been made concerning the phylogenetic relationships among the subfamilies from different viewpoints (Matsubayashi and Katakura, 2009). Phenotypic plasticity and polymorphism make species identification of ladybird beetles more difficult, sometimes even for a trained taxonomist (Marin *et al*. 2010). In these situations, morphological characteristics are so limited that species misidentifications become inevitable. DNA barcoding approach, first proposed by Hebert in 2003, has proven to be an effective tool for rapid species identification (Hebert *et al.* 2003). The 50-region of the mitochondrial Cytochrome C Oxidase subunit I **(**COI) with a length of 658 bp is selected as a standard barcode for animals (Hebert *et al.* 2004). Its effectiveness and practicability have been widely recognized among insects, especially for Lepidopterans and Dipterans (Hajibabaei *et al*. 2006; Kumar *et al*. 2012).

2. Objectives

- Collection and Identification of Coccinellids on the basis of morphological and genitalic characters.
- Description of all new species (if any) and Re-descriptions of important known species of Coccinellids.
- Molecular and Phylogenetic studies (DNA barcoding) of important known species of Coccinellids.
- Preparation of Checklist of Coccinellids.

3. Review of literature

3.1 International work

In the history of Coccinellids, first remarkable work was carried out by Linnaeus (1758). He classified these beetles under Coleoptera with genus *Coccinella.* Later, Bergstrasser (1874) classified these beetles under many families of Coccinellidae. Detailed study on family Coccinellidae was carried out by Mulsant (1846-1850). He published a

monograph, entitled “Speciesdes Coleopteres Trimeres Securipalpes” which showed the classification of Coccinellids into two large groups on the basis viz. Gymnosomides and Trichosomites. This Classification was not authentic because many of the permanent characters were not taken into consideration. Later, Crotch (1874) made an excellent revision of the family Cocinellidae, treating 1341 species under 37 genera after critical examination of Mulsants collections. Further he grouped “Epilachnides” as absolutely phytophagous form and “Coccinellids” strictly as carnivorous. He established his own system of Classification. Sicard (1909) arranged a long series of Madagascan Coccinellidae. Later, tremendous work done by a scientist Dobzhansky (1924) separated the tribe Stethorini from the Scymnini for the reception of single genus *Stethorus* Weise on the basis of female genital organs. Chapin (1926) redefined the tribe Telsimiini Casey. Li and Crook (1961) reported Epilachninae of Taiwan. The genus *Synonychimorpha* was established by Miyatake (1969) with *S. Sexpunctata* Miyatake from China as the type species.

Rao and So (1967) explored and reported ten Coccinellid species from Hong Kong. Iperti and Paoletti (1999) studied the biodiversity of predatory Coccinellid beetles and their economic importance in france. About 90% of Coccinellid beetles are considered beneficial because of their predacious nature and are also regarded as bioindicators. Some Coccinellids show migratory behaviour during unfavorable conditions but most of them withstand this hostile environment by entering into a temporary dormant stages hibernation, aestivation and aestivohibernation. (Majka *et al.* 2007) newly reported *Hyperaspis brunnescens* dobzhansky from Scatarie Island, Nova Scotia. *Hyperaspis brunnescens* dobzhansky is a rare and little known ladybird beetle which feeds on scale insects and mealybugs. (Mayadunnage *et al.* 2007) conducted a survey on predatory Coccinellid beetles in Srilanka. A total of 2682 Coccinellids from different vegetable crops were collected, belonging to 15 species, 12 genera, four tribes and three subfamilies. (Foley *et al.* 2009) presented first occurrence records of the multi-coloured Asian lady beetle, *Harmonia axyridis* from Montana and reported role of this beetle in biological control. Pang and Slipinski (2010) studied the revision of the Australian Coccinellidae *Diomus* Muslant. The genus *Diomus* Mulsant treats 30 species, mostly with distinct colour pattern, that can be identified using external characters. Wang and Chen (2010) presented the description of eight new species of the genus *Nesolotis* from China. (Wang and Ren, 2011) reported the new genus *Pangia belonging* to the tribe *Serangiini* from China. A new species *P. lamelliforma* was added to the genus, based on the combination of the numbers of tarsal and antennal segments. (Ullah *et al.* 2012) reported morphological characteristics of ladybird beetles collected from district Dir

Lower, Pakistan. Identification of these beetles showed that 14 different species in 12 genera belonging to sub-families Coccinellinae, Chilocorinae, Scymninae and Epilachninae are present in the selected area. Out of the 14 species from Dir Lower, two species (*Hypraspis rahatiana* and *Cryptogonus faizihanum*) were reported for the first time from Pakistan and is therefore, a new addition to Coccinellid fauna of Pakistan. Yi and He (2013) reported two new species of *Epilachna* from Yunnan, China viz. *Epilachna nodaodea* and *Epilachna lingulatus.* (Celli *et al.* 2021) worked on the taxonomic study on immatures of Southern Brazilian Coccinellini species providing an identification key useful in biological programme.

Few studies attempted to reconstruct the Phylogeny of the whole group rather than making intuitive reconstructions (Sasaji, 1968; Kovar, 1996) or preliminary morphological and cladistic analysis (Yu, 1994) and suggested studies based on morphological characters, the paraphyly of the Coccidulinae and Symninae. (Kobayashi *et al.* 1998) worked on molecular phylogeny of 12 Asian species of Epilachninae and determined a nucleotide sequence of a gene (cyt-oxydase-I) having 1000 bp from mitochondrial region. Molecular phylogenic tree was assembled for 10 *Henosepilachana* species using two *Epilachna* species as outgroup and taxonomic issues were discussed on the basis of suggested phylogenetic tree. According to (Herbert *et al.* 2003 and Schindel & Miller, 2005) specific DNA sequence of all living organisms can be used as identification tool which was known as DNA barcode. A specific mitochondrial gene cytochrome oxidase I (COI) can serve as the core of a global bioidentification system for animals. (Kergoat *et al.* 2004) studied on molecular phylogeny of European seed beetles (Bruchidae) using partial sequencing of three mitochondrial genes, 12S rRNA, cytochrome b, and cytochrome c oxidase subunit I. Phylogenetic relationship between different species was studied. (Fu and Zhang, 2006) was the pioneer of molecular systematic analysis of family Coccinellidae. (Foottit *et al.* 2008) tested the effectiveness of DNA barcode for identification of 300 different aphid species from more than 130 genera. A 658-bp fragment of mitochondrial DNA from the 5′ region of the mitochondrial cytochrome oxidase 1 (COI) gene was adopted as standard DNA barcode. Most of species were differentiated and sequence variation within species was low, averaging just 0.2%.

A recent molecular phylogeny of 20 species in the family Coccinellidae, based on the 18S and 28S rDNA genes, indicated that the Scymninae and Chilocorinae are paraphyletic, although there is incongruence between analysis and low statistical support (Robertson *et al.* 2008). The Phylogenetic relationships of the three major species groups of Coleopteran were inferred using the simultaneous analysis of 642 bp of the most conserved part of

mitochondrial DNA (mt DNA) cytochrome oxidase I (COI) (Bernhard *et al.* 2009). (Seago *et al.* 2011) conducted the first simultaneous Bayesian analysis of morphological and multi-locus molecular data for ladybird beetles. They mentioned that addition of morphological data significantly improve phylogenetic resolution and provided support for early diverging lineages, thereby better resolving evolutionary relationships than either data type alone.

Sayeda (2016) studied genetic diversity of two Coccinellids, *Cocinella undecimpunctata* L. and *Hippodamia variegata* (Goeze). The Study included partial sequencing of three mitochondrial genes, 12S, 16S ribosomal gene and COI gene. Phylogenetic tree of sequences was constructed by using three analytical methods, Maximum parsimony, Neighbor-joining and Maximum – likelihood. The pair-wise genetic distances (D) among the studied populations ranged from 0.0013 - 0.0041 in *Coccinella undecimpunctata* and from 0.0048 - 0.0128 in *Hippodamia variegata.* From this study it was concluded that both Coccinellids have low genetic variability. (Halim *et al.* 2017) presented first ever DNA barcode of eight Coccinella species from Malaysia. These all Coccinellid species were collected from different crops of families Solanaceae, Moraceae, Malvaceae, Convolvulaceae and Rutaceae. The association between different coccinellids were anticipated by using phylogenetic analysis of Neighbor Joining (NJ), Maximum Parsimony (MP), and Bayesian Inference (BI) by taking *Cassidinae* sp. as an out-group. (Madihah *et al.* 2017) represented a vital and comprehensive first ever DNA barcode of eight ladybird species from Malaysia. A noteworthy work by (Zang *et al.* 2018) was done on DNA barcoding of ladybird beetles and analyzed the phylogenetic relationship within the family Coccinellidae by Bayerian-Inference (BI) method. The phylogenetic analysis confirmed the monophyly of Coccinellidae, Sticholotidinae, Epilachninae and the paraphyly of Coccidulinae, Chilocorinae and Symninae.

A study based on the combined (molecular and morphological data) and phylogenetic analysis was conducted on tribe Chilocorini by (Weijing *et al.* 2020) which suggested a new classification of Chilocorini comprising 22 genera, the genus *Chilocorellus* being excluded from Chilocorini. (Huang *et al.* 2020) carried an integrative minibarcoding framework of ladybird beetles and 104 full DNA barcode sequences were obtained in which 101 barcodes were reported for the first time. A barcode gap 3% was given as appropriate genetic distance threshold to delimit species of Coccinellids through DNA barcodes.

3.2 National work

A distinguished taxonomic task was carried out by Kapur (1946). He gave a revision of the genus *Jauveria* Motschulsky. *Cryptolemus montrouzieri* Mulsant species was reported

by (Puttarudriah *et al*. 1952) in Banglore, South India. This ladybird beetle was imported in 1892 by Albert Koebele into California from Australia to attack the citrus mealybugs. Rawat and Modi (1969) reported nine species of predacious Coccinellids feeding on aphids and mite pests in Jabalpur, Madhya Pradesh, India. (Shantibala and Singh, 1991) reported 38 species of Coccinellids from Manipur, 8 species from Nagaland, 35 species from Arunachal pradesh, 16 species from Mizoram and 49 -species from Meghalaya in the northeast region of India. (Singh and Sanjeev, 2000) studied the association of Coccinellid predators with the mustard aphid, *Lipaphis erysimi* (Kalt), infesting mustard crops. This study revealed the presence of five coccinellid species from mustard crops. Out of 5 species, two were important aphidophagous Coccinellid predators of the mustard aphid.

A distinguish genus reviewed by Poorani (2001) was *Pseudaspidimerus* from the Indian subregion with description of a new species. *P. mauliki* and *P. flaviceps* were recorded for the first time from India. (Joshi and Sharma, 2008) collected 4382 adults belonging 26 species, out of which 14 were recorded first time belonging to sub-family Coccinellinae and tribe Coccinellini. A significant work was carried out by (Joydeb *et al*. 2013) on diversity, distribution and preference of predacious Coccinellids in agro and forest habitats of Tripura, north east India. A total of 1627 individuals of Coccinellids representing 24 species under 17 genera were reported. Harit (2015) observed diversity of Coccinellid fauna at various ecosystems in Champhai district of Mizoram state, north east India. A total of 13 species of Coccinellids belonging to 3 sub families were reported. (Ankalgi and Murali, 2016) reported the occurrence of 12 species of Coccinellidae belonging to 4 different subfamily and 9 genera. Out of these subfamilies, Coccinellidae was found dominant with 6 species and followed by subfamily Scymninae with 3 species while subfamily Chilocorinae and Epilachninae each with 1 species.

Very limited Molecular work on ladybird beetles has been done in India. Despite its agricultural importance, little information is available on the molecular ecology and evolution. Few attempts were done by Indian authors like (Ghosh *et al*. 2017) performed molecular characterization of ladybird beetles, predators of aphid pests in north east, India. The cytochrome oxidase I (COI) gene sequences were used to study within species genetic variation in four species of ladybird predators. The study revealed that the three most common species of ladybird predators of aphid pests in north east India are fairly homogenous with respect to the COI gene, but species of *Micraspis* are genetically diverse.

(Karthika *et. al* 2017) studied DNA barcoding to explore the genetic diversity and intra population polymorphisms of *Henosepilachna vigintioctapunctata* in South India. The study

reported the genetic diversity and intra population polymorphisms of *Henosepilachna vigintioctapunctata* populations attacking Solanaceae species in Asian Countries. A significant work was done by (Sarker *et.al* 2019) on feeding potentiality of *Coccinella septumpunctata* on three different aphid species and also conducted a study on its molecular characterization. The study revealed that this ladybird beetle showed high predation efficiency especially on three detrimental aphid species and may be useful for attacking any biocontrol program against the aphid pests. Similarly, (Boopathi *et. al* 2020) studied on life tables, consumption rates, molecular characterization and field evaluation on ladybird beetle *Harmonia sedicimnotata.* The study revealed that *Harmonia sedicimnotata* is a good biocontrol agent that could be used in an inoculative release (40 beetles / $100m^2$) to control cotton aphid, *Aphid gossypii.*

3.3 Regional work

Khan *et al.* (2009) surveyed on predatory Coccinellids in Srinagar, Kashmir valley during 2006-2007. 15 species were identified 11 belonging to the subfamily Coccinellinae. (Tara and Feroz, 2009) studied the biodiversity of coccinellid beetles in Kargil region during the years 2007-08. Five species of Coccinellid beetles were recorded and identified as *Halyzia* sp., *Adalia* sp., *Coelophora* sp., *H. variegata* (Goeze) and *C. septempunctata* (Linnaeus). (Shah and khan, 2014) studied on the assessment of Coccinellid biodiversity under pesticide pressure in Horticulture ecosystems of kashmir valley during the cropping season of 2011. The study revealed the presence of 17 and 15 species of predaceous Coccinellids in fruit and vegetable ecosystems respectively, with the biodiversity indices which indicated appreciable effect of pesticide application on the Coccinellids assemblage.

Kundoo *et al.* (2018) provided a taxonomic re-description of the species of genus *Coccinella* (Coleoptera: Coccinellidae) from Jammu and Kashmir, India. The focus was laid on detailed taxonomy of three Coccinellid species; *Coccinella septumpunctata, Coccinella undecimpunctata* and *Coccinella transversalis.* (Razia rasheed and A. A. Buhroo, 2018) conducted a study on diversity of Coccinellids from Kashmir. The results revealed that 1567 specimens of ladybird beetles were collected and identified into three sub families, 11 genera and 13 species. The diversity indices showed good diversity and rich fauna of Coccinellids. (Maqbool *et al.* 2020) carried out a preliminary survey on ladybird beetles (Coleoptera: Coccinellidae) in unmanaged apple orchard ecosystem of Kashmir Himalaya. Altogether, 12 species were recorded from Shopian with lowest species richness and Srinagar with highest.

4. Research Gaps

a) Keeping in view, the scientific and agricultural importance of ladybird beetles a comprehensive study on the Coccinellids of Jammu and Kashmir is necessary required to have the thorough fresh documentation of Coccinellid fauna.

b) Proper diversity of ladybird beetles is not known in Jammu and Kashmir.

c) DNA barcoding approach has proven to be effective tool for rapid species identification. In Jammu and Kashmir no work on molecular identification and Phylogenetic relationships has been carried out.

d) Proper Checklist of ladybird beetles in Jammu and Kashmir is not available.

e) Therefore, thorough collection and study of Coccinellids from Jammu and Kashmir is necessary so that the potential Coccinellid predators can be utilized in future biological control programmes.

5. Material and Methods

5.1 Method of the field survey and collection

The collection of Coccinellid beetles will be carried out in apple orchards, vegetable crops, mustard fields, nettle leafs and Salix species from different districts of Jammu and Kashmir. Collection will be done during day time, preferably in morning and evening hours. The ladybird beetles will be collected by hand picking and net sweeping method from different selected areas. All specimens will be preserved in 70% alcohol which will be sorted out with the help of forceps, seeing them under hand lens or under stereoscopic binocular microscope.

(a) Microscopic Preparations of the Specimens:- The collected material will be preserved in 70% alcohol. Clove oil will be used as a clearing agent after dehydration of the material in ascending grades of alcohol. Dissection will be carried out under stereoscopic binocular microscope with the help of fine needles and the appendages showing valuable taxonomic characters, including mouth parts, male and female genitalia etc. will be separated for mounting on the slides. The parts will be mounted in a drop of Canada Balsam and cover slip will be fixed for the preparation of permanent slides. Slides will be dried in oven at 37± 2°C for about a week. They will be examined under the stereomicroscope. Measurements of the body parts will be carried out by means of a stereoscope and transmitted-light microscope using an ocular micrometer.

(b) Identification: - The collected Coccinellid specimens will be identified upto the species level by examining them under a microscope (Stereomicroscope) and by using keys for

identification provided by different workers especially the recent and latest revisions on identification and classification of Coccinellidae.

(c) Description of species:- Detailed descriptions of all new species will be carried out, covering all the details on their colours, measurements of the different morphological, taxonomic and genitalic characters incorporating photograph of different body parts, and the details of date, locality, collected by and the material deposited etc. Re-description of some important species may also be incorporated where it will be required.

5.2 Molecular identification (DNA Barcoding) of Coccinellids by using standard barcoding protocols:

The method of DNA barcoding shall be carried out by the following steps

(1) Selection of sample: Coccinellid species will be selected from all the collected samples.

(2) Preparation of sample: The collected specimens will be preserved in 70% alcohol and will be stored at -20˚C for DNA isolation.

(3) Isolation of Genomic DNA: The total DNA will be extracted from individual adult specimen. Isolation of genomic DNA from Coccinellids will be done by using suitable kit methods:-

a) Nucleospin DNA Insect Mini Kit (Macherey- Nagel, MN) or
b) DNAeasy Tissue Kit (Qiagen, Germany) following the manufacture's protocol.

(4) Quality Check of genomic DNA: Using Agarose gel electrophoresis, the Gel will be stained with Ethidium bromide and the bands will be visualized under UV light.

(5) Amplication of DNA by Polymerase Chain Reaction (PCR): If the concentration of genomic DNA is desirable, the desired fragment of DNA i.e. COI will be amplified by PCR.

Requirements

- Genomic DNA
- PCR buffer
- dNTPs
- Forward and reverse primer (Standard and Suitable barcoding primers will be used for COI gene detection (Magro *et. al* 2010) or Universal DNA primers (Folmer *et al.* 1994)
- Taq Polymerase
- Nuclease free water

Steps for PCR reaction

a) **Denaturation**:- Genomic DNA sequence will be denatured at 94-96˚C into two separate strands. Separation will occur due to rising of temperature.
b) **Annealing of Primers**: Primers will bind to their complementary sequences on either side of the target sequence at a particular annealing temperature.
c) **Extension:** Taq DNA polymerase will bind and extend a complementary DNA and a new DNA strand will be produced using template by addition of nucleotides at 3' end of each primer.

(6) Analysis of PCR products by Gel Electrophoresis: Amplification and specificity of amplicon obtained in the PCR reaction will be analyzed using agarose gel electrophoresis. The gel will be visualized on UV illuminator and photographed on Gel Documentation System (BIO RAD). The final PCR product of 648bp will be obtained.

(7) DNA sequencing: All the purified segments will be directly subjected to sequences (outsourced) at specific lab for proper sequencing.

(8) Submissions to GenBank: To get the accession numbers, sequences will be submitted to the GenBank database using the sequence submission tool, BankIt and their respective accession numbers will be obtained.

(9) Phylogenetic analysis: In molecular phylogenetics, the sequence of the common gene will be used to assess the evolutionary relationship of species. The evolutionary relationship obtained through this analysis is generally a tree-like branched diagram called a phylogenetic tree. To construct a reliable phylogenetic tree, sequence alignment is the most important step which will be done by using various softwares. In the present study, phylogenetic analysis will be performed in MEGA 6.06 (Taimura *et al.* 2013).

Checklist: Preparing the checklist of all known species from Jammu and Kashmir.

6. Expected outcome

Taxonomic study of the family Coccinellidae, will be very useful for the correct identification of Coccinellid beetles. Outcome of this study can be widely utilized in taxonomy, identification, description and phylogenetic relationships of the ladybird beetles which is urgently needed for the application in Integrated Pest Management. Collected data and information generated during the course of study will provide a consolidated account of family Coccinellidae, inhabiting in various districts of Jammu and Kashmir.

7. Present proposed research programme will illuminate following points

(1) New species: There is great scope of collection and description of new species, moreover molecular characterization increases the scope of discovery of new species.

(2) New records: Extensive exploration and collection of Coccinellids will give several new records of Coccinellids from Jammu and Kashmir.

(3) Re-description of available Coccinellid species: Re-description of Coccinellid species with the details of their genitalic studies will be helpful in correct identification of Coccinellid species from Jammu and Kashmir.

(5) Phylogenetic relationships: As a promising tool to identify and discover new species, DNA barcoding might offer significant potential for identification, taxonomy and phylogeny of ladybird beetles with an aim to quantify the extent of genetic variation, if any, in different populations of ladybird beetles collected from different habitats.

8. References

Ankalgi, S. and Murali, J. (2016)**.** Diversity and distribution of Coccinellidae (Coleoptera) in Ankalga village (Gulbarga district) Karnataka, India. *International Journal of Basic and Applied Sciences.* **5 (**1): 1-5.

Beeson, C. F. C. (1941). The ecology and control of the India and the forest insects of neighbouring countries. *Reprint.* Govt of India. 767.

Bergstrasser, J. A. (1874). *Bol. Acad. Univ.* Cordova **111**: 287.

Bernhard, D., Ribera, I., Komarek, A. and Beutel R. G. (2009). Phylogenetic analysis of Hydrophiloidea (Coleoptera : Polyphaga) based on molecular data and morphological characters of adults and immature stages. *Ins. Sys. Evol.* **40**: 3–14.

Bianchi, F. J., and Van der Werf W. (2003). The effect of the area and configuration of hibernation sites in the control of aphids by *Coccinella septumpunctata* (Coleoptera : Coccinellidae) in agricultural landscapes: a simulation study. *Environ Entomol.* **32**: 1290-1304.

Boopathi, T., Singh, S.B., Dutta, S.K., Dayal, V., Singh, A.R., Chowdhury, S., Ramakrishna, Y., Aravintharaj, R., Shakuntala, I. and Lalhruaipuii, K., (2020). *Harmonia sedecimnotata*(F.): Predatory potential, biology, life table, molecular characterization, and field evaluation against *Aphis gossypii* Glover. *Scientific reports* **10**: 3079.

Celli, N. D. G. D. R., Almeida, L. M., Basílio, D. S., & Castro, C. F. (2021). The way to maturity: Taxonomic study on immatures of Southern Brazilian Coccinellini (Coleoptera: Coccinellidae) species important in biological control. *Zoologia* **38**:1-18.

Chapin, E. A. (1926). On some Coccinellidae of the tribe Telsimiini with descriptions of new species. *Proc. biol. Soc.* Washington. **39**: 129-134.

Crotch, R. (1874). A review of the Coleopterous family Coccinellidae: 1-311 pp.

Dobzhansky, T. (1924). Die Weiblichen Generationsorgane der Coccinelliden als Artmerkmal betrachtet (Coleoptera). *Ent. Mitt.* **13:** 18-27.

Foley, I. A., Ivie, M. A. and Denke, P. M. (2009). The First state record for multi-coloured Asian lady beetle, *Harmonia axyridis* (Pallas) from Montana. The Colepterists Bulletin. **63**(3): 351- 352.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytchrome c oxidase subunit one from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* **3**(5), 294-299.

Foottit, R. G., Maw, H. E. L., Von Dohlen, C. D. and Hebert, P. D. N. (2008). Species identification of aphids (Insecta: Hemiptera: Aphididae) through DNA barcodes. *Molecular Ecology Resources.* **8**(6): 1189.

Fu, J. and Zhang Y. C. (2006). Sequence analysis of mt DNA – COI gene and Molecular Phylogeny of twenty species of Coccinellids (Coleoptera: Coccinellidae). *Entomotaxo.* **28**(3): 179-185.

Ghosh, S., G. T. Behere and B. K. Agarwala (2017). Molecular characterization of ladybird predators (Coleoptera: Coccinellidae) of aphid pests (Homoptera: Aphididae) in North East India. *Current science.* **113**(9) 1755-1799.

Giorgi, J. A., Vandenberg, N. J., McHugh, J. V., Forrester, J. A., Slipinski, S. A., Miller, K. B.,

Shapiro, L. R and Whiting, M. F. (2009). The evolution of food preferences in Coccinellidae. *Biol. Control*. **51**: 215–231.

Hajibabaei, M., Janzen, D. H., Burns, J. M., Hallwachs W and Hebert, P. D. (2006). DNA Barcodes distinguish species of tropical Lepidoptera. *Proc Natl Acad Sci* USA. **103**: 968- 971.

Halim, M., Aman-Zuki, A., Mohammed, M. A. and Yaakop, S. (2017). DNA barcoding and relationships of eight ladybugs species (Coleoptera: Coccinellidae) that infesting several crops from Peninsular Malaysia. *Journal of Asia-Pacific Entomology*. **20**(3): 814-820.

Harit, D. N. (2015). Exploration of Coccinellid (Coleoptera : Coccinellidae) fauna of different Ecosystems in Champhai District of Mizoram State, North East India. *Research Journal of Agriculture and Forestry Science*. **3**(5): 21-24.

Hawkeswood, T. (1987). Beetles of Australia. *Angus and Robertson*, Sydney, Australia.

Hebert, P. D., Cywinska, A and Ball S. L. (2003). Biological identifications through DNA barcodes. *Proc Biol. Sci*. **270:** 313–321.

Hebert, P. D., Stoeckle, M. Y., Zemlak T. S and Francis, C. M. (2004). Identication of birds through DNA barcodes. *Plos Biol*. **2**: 312.

Honk, A. and Hodek, I. (1996). Ecology of Coccinellidae. *Kluwer Academic Publishers.* Boston. 464.

Huang, W., Xiufeng, X., Lizhi, H., Xinyue L., Xingmin W. and Xiaosheng C. (2020). An integrative DNA barcoding framework of ladybird beetles (Coleoptera: Coccinellidae). *Scientific Reports Nature Research* **10**: 10063.

Hunt, T., Bergsten, J., Levkanicova, Z., Papadopoulou, A., John, O.S., Wild, R., Hammond, P. M., Ahrens, D., Balke, M. and Caterino, M.S. (2007). A comprehensive phylogeny of Beetles reveals the evolutionary origins of a Super radiation. *Science.* **318** (5858): 1913- 1916.

Iperti, G. and Paoletti, M. G. (1999). Biodiversity of Predaceous Coccinellidae in relation to bioindication and economic importance. Special issue: Invertebrate biodiversity as bioindicators of sustainable landscapes. *Agriculture-Ecosystems-and-Environment.* **47:** 323–42.

Joshi, P. C. and Sharma, P. K. (2008). First records of Coccinellid beetles (Coccinellidae) from the Haridwar, (Uttarakhand), India. *Tropical Natural History*. **8**(2): 157-167.

Joyeb, M., Partha, P. B. J. and Basant, K. A. (2013). Diversity, distribution and habitat preference of predacious Coccinellids (Coleoptera: Coccinellidae) in agro and forest habitat of Tripura, northeast, India. *International Journal of Current Research.* **5**(05): 1060-1064.

Kapur, A. P. (1942). Bionomics of some Coccinellidae predaceous on aphids and coccids in North India. *Indian* J. E. **7**(4): 490.

Kapur, A. P. (1946). A revision of genus Jauravia Motschulsky (Coleoptera: Coccinellidae). *Mag. Nat. Hist. Ser.* **1113**: 75-92.

Karthika, P., Chithravel, V., Natraj, K., Kadarkarai, M., Marcello, N., Angelo, C., and Giovanni, B. (2017). Contrasting genetic diversity and intra-population polymorphism of the invasive pest *Henosepilachna vigintioctopunctata* (Coleoptera: Coccinellidae) : A DNA barcoding approach. *Journal of Asia-Pacific Entomology*. **20**: 23-29.

Kergoat, G. J., Delobel, A. and Silvain, J. F.(2004). Phylogeny and host-specificity of European seed beetles (Coleoptera, Bruchidae), new insights from molecular and

ecological data. *Molecular Phylogenetics and Evolution.* **32**(3): 855-865.

Khan, A. A., Zaki, F. A., Khan, Z. H. and Mir, R. A. (2009). Biodiversity of predacious ladybird beetles (Coleoptera: Coccinellidae) in Kashmir. *Journal of Biological Control.* **23(**1): 43- 47.

Khan, A. A. (2010). Stage-specific functional response of predaceous Ladybird beetle, *Harmonia eucharis* (Mulsant) (Coleoptera : Coccinellidae) to green apple aphid, *Aphis pomi* DE Geer (Hemiptera : Aphididae). *Journal of Biological Control.* **24**(3): 222-26.

Kobyashi, N., Tamura, K., Aotsuka, T. and Katakura, H. (1998). Molecular phylogeny of twelve Asian species of Epilachnine ladybird beetles (Coleoptera : Coccinellidae) with notes on the direction of host shifts. *Zool. Sci*. **15**(1): 147-151.

Koch, R .L., Venette, R. C. and Hutchinson W. D. (2005). Influence of alternate prey on predation of monarch butterfly (Lepidoptera: Nymphalidae) larvae by the multicolored Asian lady beetle (Coleoptera : Coccinellidae).*Environ Ecol.* **34**: 410-416.

Kundoo, A. A., Khan, A. A., Ishtiyaq A., M. A. Chatoo., N. A. Bhat and Khalid R. (2018). Taxonomic redescription of the species of Genus *Coccinella* (Coleoptera : Coccinellidae) from Jammu and Kashmir, India. *Journal of Pharmacognosy and Phytochemistry.* **7**(5): 79-82.

Kovar, I. (1996). Phylogeny. In: Hodek, I., Honk, A. Editors. *Ecology of Coccinellidae.* Dordrecht: luwer Academic Publishers: 19–31.

Kumar, N. P., Srinivasan, R and Jambulingam, P. (2012). DNA barcoding for identification of sand flies (Diptera: Psychodidae) in India. *Mol.Ecol.Resour*. **12**: 414–420.

Li, J. and Cook, F. E. (1961). The Epilachninae of Taiwan (Coleoptera.: Coccinellidae). *Pac. Insects*, **3** (1): 31-91.

Linnaeus, C. (1758). Systema naturae per regna tria naturae.secumdum classes, ordines genera, species cum characteribus differendiis, synonymis, locis, Editiodecima, reformata, *Tom. laurentii Salvii, Holmiae* **1**: 1-824

Madihah, Halim., Ameyra, A. Z., Muhamad, A. M., Salmah, Y. (2017). DNA barcoding and relationships of eight ladybugs species (Coleoptera: Coccinellidae) that infesting several crops from Peninsular Malaysia. *Journal of Asia-Pacific Entomology.**20:*** 814-820.

Magro, A., Lecompte, E., Magné, F., Hemptinne, J. L. and Crouau, R. B. (2010). Phylogeny of ladybirds (Coleoptera: Coccinellidae): are the subfamilies monophyletic.

Molecular Phylogenetics and Evolution. **54**(3): 833-848.

Majerus, M. E. N. (1994). *Ladybirds.* Harper Collins London, 359 pp.

Majka, C., Aikens, K. R., Mac Donald, A. A., Townsend S. M. and Mc Corquodale, D. B. (2007). *Hyperaspis brunnescens* Dobzhansky newly recorded in Canada from Scatarie Island, Nova Scotia. *Entomological News*. **118** (4): 402-406.

Maqbool, A., Sumi, U. A., Shahid, A. A. and Aijaz A. W. (2020). Preliminary Survey of Ladybird Beetle Composition (Coleoptera: Coccinellidae) in Unmanaged Apple Orchard Ecosystems of Kashmir Himalayas. *Proceedings of the Zoological Society,* Springer 1-15.

Maredia, K. M, Gage, S. H, Landis, D. A. and Scriber, J. M. (1992). Habitat use patterns by the seven-spotted ladybird beetle (Coleoptera: Coccinellidae) in a diverse agricultural landscape. *Biological control.* **2**: 159-165.

Marin, J., Crouau, R. B., Hemptinne, J. L., Lecompte, E., and Magro A. (2010). *Coccinella septumpunctata* (Coleoptera : Coccinellidae) a species complex. *Zool Scr*. **39:** 591-602.

Matsubayashi, K. W., and Katakura, H., (2009). Contribution of multiple isolating barriers to Reproductive isolation between a pair of phytophagous ladybird beetles. *Evolution.* **63**: 2563–2580.

Mayadunnage, S., Wijayagunasekara, H. N. P., Hemachandra, K .S. and Nugaliyadde, L.(2007). Predatory coccinellids (coleoptera: cocinellidae) of vegetable insect pest: A survey in mid country of Sri Lanka. *Tropical Agricultural Research.* **19**: 6977.

Mishra, A. K. and Yousuf, M. (2019). Notes on coccinellid beetles (Coleoptera: Coccinellidae) from forest ecosystem of Uttarakhand, India. *Journal of Biological Control.* **33**(1): 1-6.

Miyateke, M. (1969). The east Asian Coccinellid beetles presented in California academy of sciences (Tribe: Chilocorini). *Mem. coll. Agri. Echime Uni*. **14** (3):304-338.

Moreton, B. D.(1969). Ladybirds and spider mites. In: Beneficial insects and mites. Her Majesty, Stationary Office London. Ministry of Agriculture, Fisheries and Food. *Bulletin*, **20**: 15- 20.

Mulsant, M. E. (1846). Securipalpes. *Histoire Naturelle des Coleopteres de France*. **4**: 1-280.

Mulsant, M. E. (1850). Species des Coleopteres trimeres securipalpes. *Ann. Soc. Agric. Lyon.* **2**(2): 1-1104.

Obrycki, J. J. and Kring, T. J. (1998). Predaceous Coccinellidae in biological control *Annu. Rev Entomol*. **43**(1): 295-321.

Omkar and Parvez, A. (2016). Chapter 9 – lady bird beetles. Dlm. (pnyt.). In: Ecofriendly pest management for food security. *Academic Press, San Diego*. Pp. 281- 310.

Pang, H. and Slipinski, A. (2010). Revision of the Australian Coccinellidae, Genus *Diomus* Mulsant Part 2. *Annales Zoologici.* **60**(4): 493-545.

Poorani, J. (2001). A review of genus *Pseudaspidimerus* Kapur from the Indian subregion with description of a new species. *Oriental Insects*. **35** (1): 299-310.

Puttarudraiah, M., Channabasavanna, G. P. and Krishnamurty, B. (1952). Discovery of *Cryptolaemus montrouzeiri* Mulsant (Coccinellidae: Coleoptera Insecta) in Bangalore, South India. *Nature.* **169**: 377-378.

Rao, V. P. and So, P. Y. (1967). Notes on coccinellidae of Hong Kong. Tech. Bull. No. 9. Common wealth. *Institute of Biological Control*, p 9-24.

Rawat, R. R. and Modi, B. N. (1969). Record of some predaceous beetle on coccids, aphid, and mite pest from Madhya Pradesh. *Indian Journal of Agriculture Science*. **39(**11): 1057- 1067.

R, Razia. and A. A. Buhroo. (2018). Diversity of coccinellid beetles (Coleoptera : Coccinellidae) in Kashmir, India. *Entomon* **43**(2): 129-134.

Robertson, J. A., Whiting, M. F., and McHugh, J. V. (2008). Searching for natural lineages within the Cerylonid series (Coleoptera : Cucujoidea). *Mol Phylogenet Evol*. **46**: 193–205.

Salehi, T., Pashaei, R. S .H., Mehrnejad, M. R., Shokri, M. R. (2011). Ladybirds associated with Pistachio trees in part of Kerman Province, Iran (Coleoptera:Coccinellidae). *Iranian Journal of Animal Biosystematics* **7**(2): 157-169.

Sarker, S., Abdul, J. H., and Abu, F. M. D. Aslam. (2019). Feeding potentiality of *Coccinella septumpunctata* on three different aphid species and its molecular identification based on mitochondrial COI gene sequence. **14**(4): 249-254.

Sasaji, H. (1968). Phylogeny of the family Coccinellidae (Coleoptera). *Etizenia*. **35**: 1–37.

Sayed, S. M. (2016). Molecular Diversity of the Lady Beetles, *Coccinella undecimpunctata* L. and *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae) in Saudi Arabia. *Egyptian Journal of Biological Pest Control*. **26** (2): 351.

Schindel, D. E., Miller, S. E. (2005). DNA barcoding a useful tool for taxonomists. *Nature*. 435:17.

Seago, A. E., Giorgi, J. A., Li, J. and Slipinski A. (2011). Phylogeny, classification and evolution of ladybird beetles (Coleoptera : Coccinellidae) based on simultaneous analysis of molecular and morphological data. *Mol Phylogenet Evol.* **60**: 137–151.

Shah, M. A. and Khan, A. A. (2014). Assessment of coccinellid biodiversity under pesticide pressure in Horticulture ecosystems. *Indian Journal of Entomology*. **76** (2): 107-116.

Shantibala, K. and Singh, T. K. (1991). Studies on the vertical distribution of aphidophagous Coccinellids (Coleoptera: Coccinellidae) of Manipur and Nagaland. *Journal of Aphidology*. **5**: 39-43.

Sicard, A. (1909). Revision des Coccinellides de la fauna malgache (II). *Ann. Soc. Ent. Fr.* **78**: 68-165.

Singh, N. N. and Sanjeev, R. (2000). Relative abundance of different coccinellids in mustard ecosystem. *Indian Journal of Entomology*. **62**(4): 422-426.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol.* **30**: 2725-2729.

Tara, J. S. and Feroz, M.(2009). Lady Bird Beetles (Coleoptera: Coccinellidae) From Kargil. *The Bioscan*. **4**(4): 683-688.

Ullah, R., Haq, F., Ahmad, H., Inayatullah, M., Saeed, K. and Khan, S. (2012). Morphological characteristics of Ladybird beetles collected African Journal of Biotechnology. 11 (37): 9149-9155.

Vanderberg, N. J. (2002). Coccinellidae Latreille, 371-389.In: R. H. Arnett and M. C. Thomas, (eds). American Beetles.Polyphaga :Scarabaeoidea through Curculionoidea. *Boca Raton, CRC Press*, **2**: (1807) 861.

Wang, X., and Ren, S. (2011). A new genus *Pangia* to the tribe Seragiini from China. *Pan Pacific Entomologist*. **87**(4): 287-293.

Wang, X., Ren, S., and Chen, X. (2010). The genus *Nesolotis* from China with descriptions of eight new species. *Annales Zoologici*. **60**(1): 1-13.

Wenjing, L. I., Piotr L., Hermes, E. E., Jonas, E., Lizhi, H., Xia, C., W, H., Bingxu, C., Dirk, A., Adam, S., Wioletta, T. and Xingmin W. (2020). Combined molecular and morphological data provide insights into the evolution and classification of *Chilocorini* ladybird beetles (Coleoptera:Coccinellidae). *Systematic Entomology.* **45**: 447- 445.

Yi, C. and He, Q. (2013). Two new species of Epilachna Dejean, 1837 fromYunnan, China. Entomological News, **23** (3): 163-173.

Yu, G. (1994). Cladistic analysis of the Coccinellidae (Coleoptera). *Insect Sci*. **1**: 17–30

Zheng, W., Tian, W., H, Zhu., Zi, Wang. and X, Yu. (2018). DNA bacoding evaluation and implications for phylogenetic relationships in ladybirds (Coleoptera: Coccinellidae) *Taylor and Francis Group.* **1**: 446-552